

# GLUCOSE -6- PHOSPHATE DEHYDROGENASE DEFICIENCY AND HAEMOGLOBINOPATHIES IN RESIDENT OF ARSO PIR, IRIAN JAYA

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## ABSTRACT

*Telah dilakukan penelitian tentang defisiensi glukose -6- fosfatase dehidrogenase G-6-PD dan haemoglobinopati dengan populasi 223 penduduk yang terdiri atas 102 suku Jawa dan 121 suku Irian Jaya. Enam orang dari Suku Irian Jaya, ditemukan dengan defisiensi tingkat G-6-PD. Tingkat G-6-PD pada orang-orang ini berkisar antara 4 sampai 50% dari nilai nominal minimum.*

*Ditemukan pula 5 kasus haemoglobinopati. Pada satu orang dari suku Irian Jaya ditemukan haemoglobinopati yang konsisten dengan hemoglobin Lepore-Hollandia. Tiga orang dari suku Jawa menunjukkan suatu varian hemoglobin E dan seorang dari suku Jawa lainnya menunjukkan satu varian yang konsisten dengan hemoglobin fetal.*

*Sementara penemuan ini menunjukkan adanya varian hematologi dalam populasi penelitian yang mungkin berperan dalam kerentanan terhadap malaria, tetapi persentase subyek dengan varian tidak cukup besar untuk mempengaruhi secara berarti angka transmisi malaria di dalam populasi.*

## INTRODUCTION

A variety of factors affect the level of malaria transmission in endemic areas. Some of these factors are vector density and feeding behaviour, sporozoite rate, gametocyte carrier rate, level of acquired immunity of the host population and intervention programs including spraying and drug treatment. Another factor is the 'internal environment' of the host and

is separate from acquired immunity. Innate resistance encompasses all mechanisms that help the host resist either infection or illness but which are not induced by exposure to the parasite. Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and hemoglobinopathies are genetic characteristics which may play a part in innate resistance. G-6-PD deficiency can also complicate the treat-

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Running Head: G-6-PD and Haemoglobinopathies in Resident of Irian Jaya

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ment of patients sick with malaria. Because these hematologic variants can affect the host response to and treatment of malaria, epidemiologic and immunologic studies of malaria must always take these into account.

## MATERIAL AND METHODS

**Study Population:** The study population consisted of residents of Arso PIR, a village in Irian Jaya, Indonesia. Of the 223 persons tested, 102 were of Malay stock having recently migrated to Irian Jaya from the island of Java. The remaining 121 subjects were Irianese, the native population of Irian Jaya. Forty-four were females, 179 were males. Entrance in the study was strictly voluntary and was in accordance with US Navy regulations governing the use of human volunteers in medical research (SECNAVINST 3900.39A).

**Sample Collection:** Blood was drawn from the antecubital fosse by venipuncture. One ml of blood was mixed with 0.25 ml of acid citrate dextrose solution (trisodium citrate 13.2 gm/l, citric acid 4.8 gm/l and dextrose 24.5 gm/l). The samples were stored at 5°C and transported to Jakarta for testing.

**G-6-PD Deficiency Assay Method:** Blood samples were tested using the glucose-6-phosphate dehydrogenase (G-6-PD) diagnostic kit No. 345-UV from Sigma Chemical Co. (St. Louis, MO). The

test is based on the ability of G-6-PD to reduce NADP to NADPH in the presence of glucose-6-phosphate. The amount of NADPH produced is detected spectrophotometrically at 340 nm at time zero and five minutes later. The assay was performed at 30°C so no temperature correction factor was required. G-6-PD activity is expressed as units/ $10^{12}$  RBC and as units/g hemoglobin. Normal range for G-6-PD is 146-376 units/ $10^{12}$  RBC and 4.6-13.5 units/g hemoglobin.

**Hemoglobin Assay Method:** Blood samples were analyzed using the hemoglobin electrophoresis reagent system (technical bulletin 22) produced by Gelman Instrument Co. (Ann Arbor, Mich.). Blood samples are hemolyzed then electrophoresed on cellulose acetate. Hemoglobin standards A, F, S and C (Beckman Instrument Co., Brea CA) were included in each electrophoretic analysis. At the conclusion of the analysis, the hemoglobin was stained using Ponceau Solution.

## RESULTS

**G-6-PD Deficiency:** 223 persons were tested and six G-6-PD deficient subjects were found. All six were Irianese natives (Melanesian). The level of deficiency ranged from 50% to 4% of the minimum normal level (Table 1).

Table 1, G-6-PD Decieny

| Study No. | Gender | Age | Ethnic Origin | G-6-PD<br>U/10 <sup>12</sup> RBC | G-6-PD<br>U/g HgB |
|-----------|--------|-----|---------------|----------------------------------|-------------------|
| 137L      | F      | 13  | Irianese      | 56 (38) *                        | 2.3 (50) *        |
| 196L      | F      | 27  | Irianese      | 5.8 (3.9)                        | 0.2 (4.3)         |
| 76N       | M      | 30  | Irianese      | 31 (21)                          | 0.7 (15.2)        |
| 101N      | M      | 38  | Irianese      | 6.2 (4.2)                        | 0.2 (4.3)         |
| 113N      | M      | 44  | Irianese      | 17 (11.6)                        | 0.6 (13)          |
| 182N      | M      | 20  | Irianese      | 34 (23)                          | 1.1 (24)          |

Normal range – 146-376 U/10<sup>12</sup> RBC and 4.6-13.5 U/g HgB

( ) \* = % of minimum normal

**Hemoglobin Analysis:** Cellulose acetate electrophoresis of hemoglobin samples obtained from 223 persons revealed five abnormal findings. All Irianese had unremarkable hemoglobin electrophoresis patterns except one. This adult male subject had normal A and A<sup>2</sup> hemoglobin bands but also had an additional band that migrated slightly closer to the anode than control S hemoglobin. Two adult male and one adult female Javanese subjects had additional bands that migrated slightly closer to the anode than that of hemoglobin A<sup>2</sup>. One adult male Javanese subject had an additional electrophoretic band slightly cathodal to the hemoglobin A band.

## DISCUSSION

**Glucose-6-Phosphate Dehydrogenase:** Glucose-6-phosphate dehydrogenase is an enzyme which catalyzes the conversion

of glucose-6-phosphate to 6-phosphogluconate. In the erythrocyte, most glucose is metabolized anaerobically (Embden-Meyerhoff pathway) but some is consumed in an alternate pathway, the hexose monophosphate shut. G-6-PD is the first enzyme in this pathway. NADP is produced. NADPH and reduced glutathione, which requires NADPH for its production, protect the red cell from oxidative damage. A decrease in the amount or activity of G-6-PD reduces the amount of NADPH available and renders the cell liable to oxidation.<sup>1</sup> A variety of drugs can form oxidizers such as hydrogen peroxide and G-6-PD deficient cells are less able to protect themselves from damage.<sup>2</sup>

There are more than one hundred specific variants of G-6-PD<sup>3,4</sup> but most fall within three large groups: 1) African (A), 2) Mediterranean and 3) Asian.

While the types may be differentiated by biochemical analyses, the difference of the greatest importance is the varying degree of sensitivity to drugs and the severity of hemolytic anemia that can be induced in deficient persons.<sup>5</sup>

A wide variety of drugs can induce oxidative damage with a resulting hemolytic anemia. Chief among these are the sulfones, sulfonamides and a variety of antimalarias.<sup>5</sup> The last group is probably the most important because it includes primaquine and quinocrine. G-6-PD deficient red cells make a poorer host to *P.falciparum* parasites than do normal red cells and G-6-PD deficiency is distributed throughout most of the world's malarious zones. The finding of G-6-PD deficient people in Irian Jaya, and area with hyperendemic malaria,<sup>6</sup> is not surprising. The character of their deficiency is important, however, in determining if primaquine use is advisable. Persons with A (African deficiency) can safely be treated with primaquine. One regimen<sup>7</sup> calls for 45 mg once weekly for eight weeks while another<sup>8</sup> found 15 mg once a day for 14 days induced a self-resolving anemia so mild it was detectable only in the laboratory. Persons with Mediterranean and probably also Asian varieties are far more sensitive to primaquine. Normal doses can precipitate life-threatening hemolytic crises.

Unfortunately, the specific variant a person has cannot be determined by quantitative assays performed here. To establish the variant, the enzyme must be partially purified and concentrated, its  $K_m$  for NADP and G-6-P determined,

its pH optimum measured and its electrophoretic mobility determined.

The variant or variants present in the six subjects in Arso PIR is unknown but is probably one of the Asian variants, rather than an A<sup>-</sup> variant. Because of the wide range of values found in these subjects (4% to 50% of minimum normal) one must expect varying sensitivities to primaquine and other drugs known to induce hemolysis in G-6-PD deficient persons.

**Hemoglobin:** Of the 223 persons screened for hemoglobin abnormalities, five revealed unusual electrophoretic patterns. The extra band found in the Irianese adult male has been tentatively identified as Hemoglobin - Lepore Hollandia (Hb-L<sub>H</sub>). Both electrophoretic mobility (slightly anodal to control hemoglobin S) and the racial background of the donors are consistent with Hb-L<sub>H</sub><sup>(9,10)</sup>. Hb-L<sub>H</sub> consists of normal  $\alpha$  chains combined with chains. The chains are thought to be the product of a cross-over between  $\alpha$  and chain genes during meiosis<sup>11,12</sup>. Approximately 12% of the hemoglobin in persons heterozygotic for this trait is Hb-L<sub>H</sub>. Fetal hemoglobin can often be present<sup>(9)</sup> but was not detected in this case.

Two adult male and one adult female Javanese subject had electrophoretic bands that migrated anodally to hemoglobin A<sub>2</sub>. This hemoglobin has been tentatively classified as hemoglobin E (Hb-E) on the basis of electrophoretic mobility and the racial background of the donors. Hb-E is so common in Southeast Asia, it can be considered a polymorphism<sup>11,13</sup>.

Homozygosity for Hb-E produces a mild microcytic anemia while the carrier state (30 to 40% of hemoglobin is Hb-E) is asymptomatic<sup>(11)</sup>. A more serious situation arises when the gene for Hb-E is inherited along with a B-thalassemia gene producing Hb-E thalassemia. Although this condition can present in a variety of ways, in its severe forms, it can simulate Cooley's anemia<sup>(12)</sup>

One adult Javanese male had an extra electrophoretic band consistent with fetal hemoglobin (Hb-F). This may be an example of hereditary persistence of fetal hemoglobin (HPFH). A variety of inherited conditions can result in retention of Hb-F but disease is usually absent<sup>(12)</sup>.

There is considerable overlap between the malarious areas of the world and where the highest incidences of G-6-PD deficiency and hemoglobinopathy occur<sup>14</sup>. There are indications that G-6-PD deficiency confers some protection against severe *P. falciparum* infection<sup>15,16</sup>. Hemoglobin E, however, has not yet been tied with certainty to protection against parasitemia<sup>17</sup>. If, indeed, these genetic variations do provide some protection, it is certainly not absolute<sup>18,19</sup>. Individuals with G-6-PD deficiency and hemoglobinopathies are still liable to malaria infection and disease. Only when populations, rather than individuals, are studied do differences appear. This means that in any malaria epidemiology, inborn differences in the red blood cell must be considered before the dynamics of malaria transmission in a population can be characterized.

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